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Short running head: Bone mineral density in East Greenland polar bears.

Key words: Polar bear, *Ursus maritimus*, PCBs, DDTs, CHLs, dieldrin, endocrine disruption, osteoporosis, bone mineral density, BMD.

Abbreviations:

∑	Sum of congeners
BMD	Bone Mineral Density
CHLs	CHLordanes
CYP	Cytochrome P-450 isozymes
DDD	Dichloro Diphenyl Dichloroethane
DDE	Dichloro Diphenyl Dichloroethylene
DDT	Dichloro Diphenyl Trichloroethane
DXA	Dual X-ray Absorptiometry
HCB	HexaChloroBenzene
HCHs	HexachloroCycloHexanes
HPA	Hypothalamic-Pituitary-Adrenal

PBDEs	PolyBrominated DiphenylEthers
PCBs	PolyChlorinated Biphenyls
PCDDs	PolyChlorinated DibenzoDioxin
PCDFs	PolyChlorinated DibenzoFurans
*p*QCT	*p*eripheral Quantitative Computed Tomography

Section headers:

Abstract

Bone mineral density (BMD) in polar bear (*Ursus maritimus*) skulls (n=139) from East Greenland sampled during 1892-2002 was analysed. The primary goal was to detect possible changes in bone mineral content (osteopenia) due to elevated exposure to organochlorine (PCBs, DDTs, CHLs, dieldrin, HCHs, HCB) and PBDE compounds. To ensure that the BMD in skull represented the mineral status of the skeletal system in general, BMD in femur and three lumbar vertebrae were compared in a subsample. Highly significant correlations between BMD in skull and femur (r=0.99; p<0.001; n=13), and skull and vertebrae (r=0.97; p<0.001; n=8) were detected. BMD in skulls sampled in the supposed pre-organochlorine and PBDE period (1892-1960) was significantly higher than in the supposed pollution period (1961-2002) for subadult females, subadult males and adult males (all: p<0.05) but not adult females (p=0.94). Negative correlation between organochlorines and the skull BMD was in subadults found for Σ-PCBs (p<0.04) and Σ-CHLs (p<0.03) and in adult males for dieldrin (p<0.002) and Σ-DDTs (p<0.02) (indications for Σ-PBDEs in subadults; p=0.06). In conclusion, the strong correlative relationships suggested that disruption of the bone mineral composition in East Greenland polar bears may have been caused by organochlorine exposure.

Introduction

Bone mineral composition in mammals is based on a complex set of inter-related mechanisms, and is influenced by various nutritional and environmental factors (*e.g.* Ganong 1991; Johansson and Melhus 2001; Johansson et al. 2002; Leder et al. 2001; Michaelsson et al. 2003; Promislow et al. 2002; Sarazin et al. 2000). Furthermore, environmental stressors such as exposure to harmful chemicals, starvation, temperature extremes and noise have been shown to disrupt bone mineral composition in laboratory mammals (Bergman and Olsson 1985; Brandt and Siegel 1978; Doyle et al. 1977; Feldman 1995; Mooney et al. 1985; Nilsson 1994; Siegel and Doyle 1975a, b; Siegel et al. 1977, 1992; Siegel and Mooney 1987). The pathogenesis of stress-induced bone mineral changes is an activation of the hypophyseal-adrenal/thyroid axis, leading to enhanced parathyroid and cortisol hormone secretion and increased bone resorption, while bone formation is decreased (Colborn et al. 1993, Damstra et al. 2002; Feldman 1995; Ganong 1991, Selye 1973). Other hypotheses on disruption of bone mineral status include altered mitotic rates, changes in local subcellular calcium transport or decreased protein synthesis (Siegel and Mooney 1987).

Organochlorines like PCBs (PolyChlorinatedBiphenyls), DDTs (DichloroDiphenylTrichloroethanes), CHLs (CHLordanes), HCHs (HexaCycloHexanes), dieldrin, HCB (HexaChloroBenzene), PBDEs (PolyBrominatedDiphenylEthers) and aryl hydrocarbon receptor (AhR) active organochlorines (*e.g.* polychlorinated dibenzo-*p*-dioxins, dibenzofurans and non-*ortho* chlorine-substituted PCBs) are all lipophilic (low degradable) chemicals, pesticides, or unwanted chemical by-products (*e.g.* de March et al. 1998). In general, the presence of such compounds in the arctic marine environment is the result of long-range atmospheric transport from lower latitude sources in more industrial areas of the world, where outputs and use of, *e.g.*, PCB peaked in the 1960's (de March et al. 1998). Due to their lipophilicity; organochlorines and PBDEs persist in the environment (AMAP

2004; Colborn et al. 1993; Damstra et al. 2002; de March et al. 1998). In polar bears, organochlorines are consequently transferred transplacentally from mother to fetus and via lactation, resulting in fetal and neonatal exposures that have the potential for adverse health effects, *e.g.* on growth and development (Bernhoft et al. 1997; Birnbaum 1994; Polischuk et al. 1995, 2002).

DDTs and PCBs in humans have been associated with low bone mineral density (Alveblom et al. 2003; Beard and Young 2000; Glynn et al. 2000) through their action as exogenous agonists and antagonists to naturally endogenous hormones (Damstra et al. 2002). Various organochlorines have also been linked to periodontitis and osteoporosis in marine fish and mammal wildlife (Bengtsson et al. 1985, Bergman et al. 1992, de Guise et al. 1995; Lind et al. 2003; Lind et al. 2004; Mortensen et al. 1992; Schandorff 1997a; Zakharov and Yablokov 1990) and in the laboratory (Fernie et al. 2003; Jamsa et al. 2001; Lind et al. 1999; Lind et al. 2000a, 2000b; Render et al. 2000a, 2000b, 2001; Singh et al. 2000; Valentine and Soulé 1973). In various mammalian wildlife, osteopenia and macroscopic pathology have been examined in bone during distinct periods of exposure to anthropogenic pollutants have been examined in *e.g.* grey seal (*Halichoerus grypus*), ringed seal (*Phoca hispida*) harbour seal (*Phoca vitulina*) and alligator (*Alligator mississippiensis*) (Bergman et al. 1992; Lind et al. 2003; Lind et al. 2004; Mortensen et al. 1992; Zakharov and Yablokov 1990; Schandorff 1997a, 1997b; Sonne-Hansen et al. 2002). The studies showed relationhips between organochlorines and exostosis, periodontitis, loss of alveolar bone structures, osteoporosis, widening of the canine opening and enlargement of the foramen mentalia.

Polar bears (*Ursus maritimus*) from East Greenland, Svalbard and the Kara Sea carry higher loads of organochlorines than polar bears elsewhere in the Arctic due to their reliance on blubber from ringed seal (*P. hispida*) and bearded seal (*Erignathus barbatus*) (*e.g.* AMAP 2004; de March et al.

1998; Lie et al. 2003; Norstrom et al. 1998). Recent studies of polar bears from Svalbard have indicated that high levels of organochlorines negatively affect retinol (vitamin A) and levels of thyroid hormones (Skaare et al. 2001) and possibly also negatively affect sex steroids and reproductive organs (female pseudohermaphrodites) - although these latter mechanisms are not clearly understood (Haave et al. 2003; Oskam et al. 2003; Sonne et al. in press; Wiig et al. 1998). Other studies have associated high levels of organochlorines with low levels of IgG suggesting a possible immunotoxic effects on the IgG levels (Bernhoft et al. 2000; Lie et al. 2004, submitted). Overall, these studies support the notion that organochlorines may cause disruption and thereby potentially affect bone mineral composition.

To determine whether exposure to organochlorines and PBDEs may have adversedly affected bone mineral composition in polar bears, we compared BMD in skulls of 41 individual polar bears collected in East Greenland during the supposed pre-polluted period (1892-1960) with 98 polar bear skulls collected during the supposed polluted period (1961-2002). The year 1961 was chosen as dividing year due to the transport of organochlorines (and later PBDEs) from lower latitudes to East Greenland (Norstrom et al. 1998; de March et al. 1998, AMAP 2004). Furthermore, we examined a sub-set of 58 of the individuals collected during the pollution period to determine if BMD was related to body burden of various organochlorines and PBDEs.

Materials and methods

Sampling and age estimation

A sample of 139 East Greenland polar bear skulls (sampled between Skjoldungen at 63º15'N and Danmarks Havn at 76º30'N) sampled during 1892-2002 was studied. The age determination was carried out by counting the cementum Growth Layer Groups (GLG) of the lower I_3 after decalcification, thin sectioning (14µm) and staining (toluidine blue) using the method described by *e.g.* Hensel and Sorensen (1980) and Dietz et al. (1991). For analyses, the individuals were then categorised into subadults, adult males and adult females by these criteria: adult males ≥ 6 years, adult females ≥ 5 years and others as subadults (*e.g.* Rosing-Asvid et al. 2002). Regarding skull samples from 1892-1987 the sex was available from the expedition files, and in case of absence of this information (*n*=9) the determination was based on skull morphology.

Osteodensitometry

X-ray osteodensitometry was applied to detect osteopenia (osteoporosis) by use of a Norland XR 26 X-ray bone densitometer (Norland Corporation, Wisconsin, USA) which determined the bone mineral density (calcium-phosphate; hydroxyapatite) during a dual X-ray absorptiometry (DXA). The skulls were scanned in "*Research*" mode (speed: 60 mm/sec; resolution: 3.0 x 3.0 mm; width: 24,9 cm) and analysed in XR software revision 2.4®, which generated a picture of the bone segment and calculated the bone mineral density of hydroxyapatite (BMD; g cm^{-2}) (*Ibid.*) (Fig. 1).

To ensure that BMD in the skull represents the mineral status of the skeletal system in general, a study was conducted where the BMD of the skull, one femur and three lumbar vertebrae were compared in a sub-set of 13 polar bears (3 subadults, 2 adult females and 8 adult males) from the Co-

penhagen Zoo and East Greenland. The DXA-scanner was daily calibrated using a phantom with known mineral density. In addition the precision was tested by a 10 time rescanning (mean=521.96 g cm^{-2}, SD=0.60) which from the formula [1 – (SD/mean) x 100%] gives a precision of 99.88%. Fragmentation and loss of teeth material caused by handling and lead shot was thought to be a problem. A double determination of the BMD in 2 skulls (#5483 and #2891) with and without incisors, canines, premolars and molars showed that loss of half or more of the material of the large canines altered the result significantly. As none of the canines in the entire material were fragmented to such a degree, fragmentations were not considered a problem.

Contaminant analyses

Polar bear subcutaneous adipose tissue samples (*n*=58) were analysed for PCBs DDTs, HCHs, CHLs, HCB, dieldrin and PBDEs as described elsewhere (Dietz et al. in press; Luross et al. 2002; Sandala et al. 2004). Sum (Σ) PCBs are the total concentrations of the 51 individual or co-eluting congeners (if detected): CB # 31/28, 52, 49, 44, 42, 64/71, 74, 70, 66/95, 60, 101/84, 99, 97, 87, 110, 151, 149, 118, 146, 153, 105, 141, 179, 138, 158, 129/ 178, 182/ 187, 183, 128, 174, 177, 171/202/156, 200, 172, 180, 170/190, 201, 203/196, 195, 194, 206. Σ-DDTs are the sum of *4,4'*-DDT, *4,4'*-DDD and *4,4'*-DDE. Σ-HCHs are the sum of the α-, β- and γ-hexachlorocyclohexane. ΣCHLs are the total concentrations of oxychlordane, *trans*-chlordane, nonachlor III (MC6), *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide. Σ-PBDE concentrations are the total of 35 individual or co-eluting congeners (if detected): BDE# 10, 7, 11, 8, 12/13, 15, 30, 32, 28/33, 35, 37, 75, 71, 66, 47, 49, 77, 100, 119, 99, 116, 85, 155/126, 105, 154, 153, 140, 138, 166, 183, 181, 190 (Muir et al. in prep.). All contaminant data is given in ng/g lipid weight (l.w.).

Statistics

The BMD showed no deviation from normality (Shapiro-Wilk test) while contaminant data was log-transformed (base e) prior to the analyses in order to meet the criteria of normality and homogeneity of the variance. The significance level was set to $p \leq 0.05$, while significance levels of $0.05<p \leq 0.10$ was considered a trend. *First*, the condylobasal skull-length versus age was tested within each group (*i.e.* subadults of both sexes, adult females and adult males) in an analyses of covariance with skull-length as dependent variable, periods (before and after 1960 respectively) as class variables, age as covariable and their 1st order interaction links (age*period). The result from this analysis showed that the skull-length vs. age relation was the same in the two periods which justified the use of non-length-corrected skull BMD in the further analyses (all: $p>0.26$). *Secondly*, the BMD versus age relationship was tested by a linear regression analyses (BMD as dependent variable and age as independent variable) for subadults of both sexes, adult females and adult males. To test for *period differences*, an ancova was used with BMD as dependent variable, age/sex (subadult females, subadult males, adult females and adult males) and period (before and after 1960 respectively) as class variables, the age as covariable and the 1st order interactions links (age*period, age*age/sex and age/sex*period) between these variables. The model was successively reduced for non-significant interactions ($p>0.05$) judged from the type-III sum of squares, and the significance of the remaining factors was evaluated from the final model (LSMean). A *temporal trend* over the entire period 1892-2002 was analysed by a multiple regression analysis with skull BMD as the dependent variable and the individual age and year of kill as explanatory variables for subadults of both sexes, adult females and adult males respectively (the relationship was evaluated from the parameter estimate, r^2 and p-value). The *relation between age/sex groups and contaminants* was analysed within a one-way anova on the log-transformed contaminant data and significant results were tested among each other by Tukey's *post hoc* test. The *Skull BMD versus contaminants* (∑-

PCBs, ∑-DDTs, ∑-CHLs, HCB, ∑-HCHs, dieldrin and ∑-PBDEs) relationships were explored by multiple regressions with skull BMD as the dependent variable and the age and contaminant concentrations as explanatory variables within age/sex groups (subadults of both sexes, adult females and adult males). Finally the relationship between levels of contaminants and BMD was evaluated from the parameter estimate, r^2 and p-value.

Results

We found a highly significant correlation between BMD (bone mineral density) in skull and femur (r=0.99; p<0.001; n=13), and skull and vertebrae (r=0.97; p<0.001; n=8). These results justified the use of BMD measurements in skull to reflect the status of the skeletal system although information on body conditions and nutritional stressors, relevant for osteoblastic and -clastic activity, was not available.

Skull BMD and age/sex differences

BMD was analysed in 139 skulls representing the period from 1892 to 2002 and consisted of 64 subadults, 40 adult females and 35 adult males. The BMD increased with age in subadults (p<0.001) but not adults (both: p>0.05) (Fig. 2). BMD differed between males and females (p<0.01) in the order: subadult females<subadult males<adult females<adult males. Furthermore, BMD in females 14-23 years of age seemed to decline significantly with age (p<0.04).

Period differences and temporal trends in skull BMD

Forty-one skulls were available from the supposed pre-pollution period (1892-1960) and 98 from the supposed pollution period (1961-2002) (Table 1). BMD in skulls sampled in the pollution period was significantly lower than BMD sampled in skulls from the pre-pollution period for subadults and adult males (p<0.05), but not for adult females (p>0.9) (Table 1). In addition, the multiple regression analyses of BMD versus individual age and year of kill (1892-2002) showed that BMD decreased over the entire period in adult males (p<0.01) and a similar trend was found for subadults (p=0.07) (Table 2). There was no BMD time trend for adult females (p>0.5).

Skull BMD and contaminants

The range and variation of organochlorine and PBDE contaminants (ng/g l.w.) in the 58 polar bears sampled during 1999-2002 are presented in Table 3. Levels of ∑-DDTs, dieldrin, ∑-HCHs and ∑-PBDEs was not different between subadults, adult females and adult males (all: $p>0.07$). But, levels of ∑-PCBs were higher in adult males when compared to adult females ($p \leq 0.05$). Further information on the relation between organochlorines and age, sex and season in East Greenland polar bears from 1999-2002 is available in Dietz et al. (in press) and Sandala et al. (2004).

BMD was found to be negatively correlated with levels of ∑-PCBs ($p<0.04$) and ∑-CHLs in subadults ($p<0.03$) while BMD was negatively correlated to ∑-DDTs ($p<0.02$) and dieldrin ($p<0.002$) in adult males (Table 4). In addition, a trend of ∑-PBDEs being negatively correlated to BMD in subadults was found ($p=0.06$) while no significant relations were found for adult females (Table 4).

Discussion

BMD and age/sex differences

The high correlation between skull BMD and femur and vertebrae, respectively, is usefull as skull samples of polar bears (and other mammals) are present at national zoological museums all over the world, which make various time-trend bone studies possible. Our results clearly showed that skull BMD increased more rapidly in subadults compared to adults in accordance with previous studies of ringed seals from NW Greenland (Sonne-Hansen et al. 2002). Female polar bears usually give birth to two cubs every third year (December) and mobilize and tranfer large amounts of calcium and phosphate during the gestation and *post partum* (suckling) period lasting for up to two years (Ramsay and Stirling 1988). In this period calcium is used for foetal skeletal production and maintenance of the mothers and her offspring's calcium-phosphate homeostasis (*Ibid.*). As the female polar bear mobilizes these large amounts of calcium and phosphate, it could be expected that adult females have a lower BMD compared to adults males. Such a difference was also found in the present study. Similiar differences have been found in humans (*e.g.* Van Langendonck et al. 2002). As suggested for humans, the marked difference in BMD between the sexes could be the result of a higher muscle mass and strength in males, leading to higher biomechanical loading of the bone. This would lead to an increased bone formation through the stimulation of mechanotransduction system in the osteocytes (*Ibid.*).

Earlier studies show that sufficient levels of sex steroids (estrogens, androgens) are important in the development of the human cortical bone structures in boys, girls, teenagers, adults and elderly (Hampson et al. 2002; Juul 2001; Leder et al. 2001; Szulc et al. 2001). On the other hand, high levels of estrogen active substances (intrinsic, extrinsic) stimulate the expression of secondary sexual

characteristics (*Ibid.*). Therefore growth delay and osteopenia (osteoporosis) have been associated with hypogonadism and lower estrogen levels in both sexes (Leder et al. 2001; Nelson 2003; Szulc et al. 2001). Indications of such age-related decrease in BMD in females was found in the present study probably associated with a menopause phase after the 15th year of age but this requires a larger sample size (Fig. 2) (Derocher and Stirling 1994).

Period differences and temporal trends in skull BMD

In both analyses of subadults of both sexes and adult males the individuals from the pre-pollution period had a higher skull BMD compared to the polluted period. These results suggest that there is a linkage between decreased BMD for bears from the polluted period, and exposure to environmental stressors compared to bears in the pre-pollution period. Two major environmental stressors could be linked to mineral loss in polar bear skulls: anthropogenic organochlorine compounds and PBDEs and/or climate oscillations (AMAP 2004; de March et al. 1998; Førland et al. 2002). Concentrations of *e.g.* ∑-PCBs in the adipose tissue of East Greenland polar bears have over the last four decades reached levels that can elicit adverse biological effects on immunological parameters and vitamin A (stress), which may be linked to the present decrease in skull BMD (AMAP 2004; de March et al. 1998). However, during the same period global warming has resulted in a reduction in the ice coverage in the East Greenland area (Comiso 2002; Rothcock et al. 1999). Although population ecology has not been studied in East Greenland, the situation is probably similar for polar bears from the Hudson Bay area in Canada (Stirling et al. 1999). A reduction of the sea ice in the Hudson Bay area has reduced the bears' access to ringed seals resulting in reduced body condition and lowered natality in the polar bears (*Ibid.*).

Temporal differences with respect to potential effects of PCB and DDT exposure on periodontitis and osteoporosis in grey seal and harbour seal was investigated by Bergman et al. (1992); Mortensen et al. (1992) and Schandorff (1997). They found exostosis and periodontitis often with substantial loss of alveolar bone in mandible and maxilla (osteoporosis). These changes could have been caused by hormonal imbalance potentially induced by PCBs and DDTs with malformation of the calcium helix structures around the collagen matrix (DeLillis 1989). These results are further supported by the investigations of Render et al. (2000a, b, 2001). However it must be noted that the range in Σ-PCB and Σ-DDT levels in the seals were orders of magnitude higher compared to levels in the present polar bears (Blomkvist et al. 1992).

Lind et al. (2003) investigated the bone mineral density (g cm^{-3}) in the male grey seals (*n*=43) reported above by Bergman et al. (1992). The method used was pQCT (*p*eripheral *q*uantitative *c*omputed *t*omography) which made it possible to distinguish between cortical and trabecular bone in *os mandibularis* and *os radius* respectively (DXA-scanning used in the present study gives the average of trabecular and cortical bone density). Three sample groups of seals were compared: 1850-1955 (no pollution); 1965-1985 (high pollution) and 1986-1997 (fairly low pollution). They found that radius trabecular bone mineral density was significantly higher in the fairly low pollution period (1986-1997) compared to the high pollution period (1965-1985) while for mandible cortical bone mineral density was significantly lower in the fairly low pollution period (1986-1997) compared to the no pollution period (1850-1955). Our study of BMD in East Greenland polar bears supports the findings of Lind et al. (2003).

BMD levels and contaminants

Bone density expresses the bone mineral composition determined by the activity of osteoblastic bone formation and osteoclastic bone resorption which is regulated by androgens and estrogens through cytokines (Manalagas and Jilka 1995; Manalagas et al. 1995). Studies on Svalbard have shown that PCB may negatively influence plasma testosterone levels (Oskam et al. 2003) and plasma retinol and thyroid hormone levels in polar bears (Skaare et al. 2001). These studies all indicate that organochlorines in Svalbard polar bears (and likely also East Greenland bears, as the OHC levels are comparable) potentially affects the endocrine homeostasis, which again may lead to bone mineral loss (osteoporosis) (*Ibid.*). Another polar bear study from Svalbard associated high levels of organochlorines with low levels of IgG suggesting possible immunotoxic effects (Bernhoft et al. 2000, Lie et al. 2004, submitted). This potential effect may lower the immune response and enhance stress with increased cortisol levels), which potentially affects the bone mineral composition (osteoporosis).

The present study indicated that high concentrations of Σ-PCBs and Σ-CHLs are associated with reduced skull BMD in subadults and that Σ-DDTs and dieldrin are associated with reduced skull BMD in adult males. These BMD relationships with Σ-PCBs, Σ DDTs, Σ CHLs and dieldrin concentrations in adult males and subadults of both sexes may suggest endocrine-related effects (*e.g.* AMAP 2002; Birnbaum 1994; Damstra et al. 2002; de March et al. 1998; Lind et al. 2003; Lind et al. 2004). For example, PCBs and DDTs have shown in vitro and vivo to be weak agonists antagonists of estrogen receptor-mediated activity, or OC-mediated induction of CYP450 activity can impact circulating sex hormone levels (*e.g.* estrogens) (Navas and Segner 1998) and this is also of relevance in the polar bear (*Ursus maritimus*) (*e.g.* Letcher et al. 1996). Relationships between 4,4'-DDE concentrations and BMD in humans have been reported (Beard and Jong 2000; Glynn et al.

2000). Glynn et al. (2000) found significant negative correlations between 4,4'-DDE and BMD in 68 sedentary women (where concentrations are lower compared to the present polar bears), and concluded that 4,4'-DDE may also have a negative effect on BMD in men (with contaminant levels comparable to those found in the polar bears). Lind et al. (2004) investigated the relationship between DDTs and bone composition in juvenile female american alligators (*Alligator mississippiensis*) in Lake Apopka. Compared to a non-polluted reference alligator subpopulation the tibial trabecular BMD was increased and the authors suggested that environmental estrogenic copounds (DDTs and its metabolites a.o.) disrupted the normal bone remodelling process (inhibition of osteoclast activity) which had resulted in increased BMD.

Guo et al. (1994) found that for primiparous PCB contaminated mothers (Yu-Cheng; rice-oil disease) their children (n=25) were significantly smaller and had less total lean mass, less soft tissue mass but not lower bone mineral density compared to a control group. The PCB levels in the children (serum) were 10.3 ng/g l.w. and were lower than the levels in the present study. Alveblom et al. (2003) investigated the incidence of osteoporotic fractures in fishermen and their wives from the Baltic Sea (high pollution) and compared these to fishermen from the west coast of Sweden (low pollution) as controls. For vertebral fractures there was a significantly higher IRR (incidence rate ratio) for east coast (Baltic) women compared to west coast women and a similiar but non-significant tendency was found for men. The PCB concentration (10 congeners) was 2000 ng/g l.w. (serum) which was significantly higher compared to the west coast population but lower compared to the range in the subcutaneous adipose tissue of East Greenland polar bears. These environmental studies support the findings of negative associations between PCBs/DDTs and BMD levels in East Greenland polar bears.

A negative correlation was observed in the present bears between ΣPBDE concentrations in adipose tissue and BMD in subadults. Disturbances in thyroid function and developmental toxicity (histopathology) have been shown to be associated with PBDEs in laboratory rats (*e.g.* de Wit 2002) as well as polar bears from Svalbard (Skaare et al. 2001).

Conclusions

Skull bone mineral density (BMD) increased with age in subadults and was higher in males than in females at all ages. For adult females older than 13 years of age a menopausal BMD decrease was indicated but a further examination requires a larger sample size. BMD in skulls from subadult females, subadult males and adult males sampled in the supposed pollution period (1961-2002) was significantly lower than BMD in skulls from the supposed pre-pollution period of organochlorine and PBDE compunds (1892-1960). Furthermore, correlative relationships suggested that Σ-PCB, Σ-CHL, dieldrin and Σ-DDT exposure negatively influenced BMD in skulls from subadults of both sexes and adult males.

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Table 1. Skull bone mineral density (BMD) for subadult and adult East Greenland polar bears from 1892 to 2002.

Period	**Variable**	**Subadult females**	**Subadult males**	**Adult females**	**Adult males**
Period 1	BMD	1.67±0.37 (7)	2.22±0.19 (5)	1.99±0.13 (9)	2.73±0.21 (20)
	Age	2.6±1.3 (7)	4.4± 1.3 (5)	12.7±3.7 (9)	11.5±4.5 (20)
Period 2	BMD	1.55±0.3* (17)	1.85±0.32* (35)	1.98±0.13 (31)	2.49±0.24** (15)
	Age	2.8±1 (17)	3.2±1.1 (35)	12.1±6.3 (31)	10.7±5.5 (15)

Data are divided on period 1 (1892-1960: supposed organochlorines and PBDEs non-polluted) and period 2 (1961-2002: supposed organochlorines and PBDEs polluted). BMD (g cm^{-2}) was obtained by DXA-skanning of the entire skull and age (years) was obtained by counting the GLG of the lower I_3 tooth . Data is given in mean±SD and number of observations is given in brackets. *: BMD in period 2 significant lower compared to period 1 for the given age/sex group at the $p \leq 0.05$ level. **: BMD in period 2 significant lower compared to period 1 for the given age/sex group at the $p \leq 0.01$ level.

Table 2. Significant results from the multiple regression analyses of skull bone mineral density (BMD) versus age and year of kill in East Greenland polar bears during 1892-2002.

Age/sex group	**Equation**	r^2	p_{age}	p_{yok}	*n*
Subadults	BMD=0.193*age–0.00254*yok+6.3	0.64	<0.001	0.07*	40
Adult males	BMD=0.014*age–0.00324*yok+8.8	0.31	0.2	<0.01**	35

The equation is given as: [BMD=A*age+B* yok+C]. Dependent variable: BMD (g cm^{-2}). Explanatory variables: Age (years) and year of kill (yok; 1892-2002). A, B and C: specific parameter estimates. r^2: regression coefficient of the model, p_{age}: p-value for age and $p_{yok:}$ p-value for year of kill. *: *i.e.* non-significant trend of BMD decline over the entire period 1892-2002 at the $0.05<p\leq0.10$ level. **: *i.e.* significant BMD decline over the entire period 1892-2002 at the $p\leq0.01$ level.

Table 3. Concentrations of various contaminants in subcutaneous adipose tissue of 58 East Greenland polar bears sampled during 1999-2001.

Compound	**Subadults (n=35)**	**Adult females (n=14)**	**Adult males (n=9)**
∑-PCBs	6597±2726 (6089)	5334±2150* (5770)	8637±4111* (8280)
∑-CHLs	1598±884 (1469)	1379±591 (1353)	1055±517 (914)
∑-DDTs	392±209 (376)	358±149 (366)	481±331 (496)
∑-HCHs	196±68 (172)	195±186 (151)	294±210 (181)
Dieldrin	210±100 (196)	174±70 (154)	177±81 (172)
HCB	99±84 (70)	75±82 (51)	51±28 (48)
∑-PBDEs	62±33 (53)	53±17 (53)	52±16 (49)

Contaminant data is given in ng/g l.w. within groups of subadults of both sexes, adult females and adult males. Data is given in mean±SD and the median in brackets. *: significant difference between adult females and males at the $p \leq 0.05$ level.

Table 4. Significant results from the multiple regression analyses of skull bone mineral density (BMD) versus age and contaminant concentrations in East Greenland polar bears sampled during 1999-2001.

Age/sex group	**Equation**	r^2	p_{age}	p_{cont}	n
Subadults	BMD=0.26*age–0.25*[Ln(∑-PCBs)]+3.1	0.59	<0.001	<0.04**	35
Subadults	BMD=0.24*age–0.19*[Ln(∑-CHLs)]+2.4	0.6	<0.001	<0.03**	35
Subadults	BMD=0.25*age–0.18*[Ln(∑-PBDEs)]+1.69	0.58	<0.001	0.06*	35
Adult males	BMD=0.02*age–0.17*[Ln(∑-DDTs)]+3.4	0.69	>0.08	<0.02**	9
Adult males	BMD=-0.005*age–0.37*[Ln(Dieldrin)]+4.5	0.85	0.43	<0.002***	9

The equation is given as: [BMD=A*age+B*Ln(cont)+C]. Dependent variable: BMD (g cm^{-2}). Explanatory variables: Age (years) and Ln-transformed contaminant concentration [Ln(ng/g l.w.)]. A, B and C: specific parameter estimates. r^2: regression coefficient of the model, p_{age}: p-value for age and p_{cont}: p-value for contaminants. *: *i.e.* non-significant trend of a negative correlation between BMD and Ln(∑PBDEs) at the $0.05<p\leq0.10$ level. **: *i.e.* significant negative correlation between BMD and organochlorine contaminant group at the $p\leq0.05$ level; ***: *i.e.* significant negative correlation between BMD and organochlorine contaminant group at the $p\leq0.01$ level.

FIGURE LEGENDS

Figure 1. DXA scanning image of a 12-year-old female East Greenland polar bear sampled in 1972. Note the high density areas of cortical bone tissue (light) and the lower density areas of trabecular bone tissue (dark).

Figure 2. BMD (g cm^{-2}) in skulls from East Greenland polar bears versus individual age (years). Subadult females: Δ, subadult males: ▲, adult females: □ and adult males: ■.

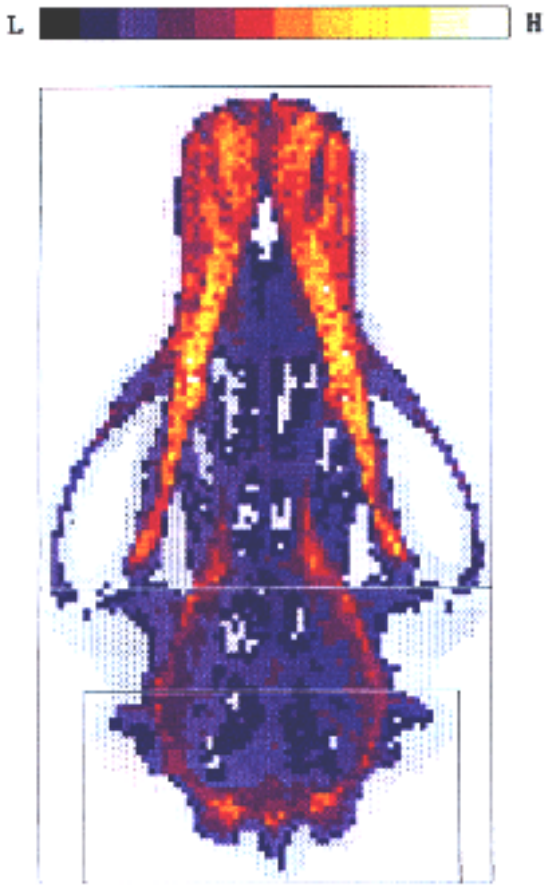

Figure 1.

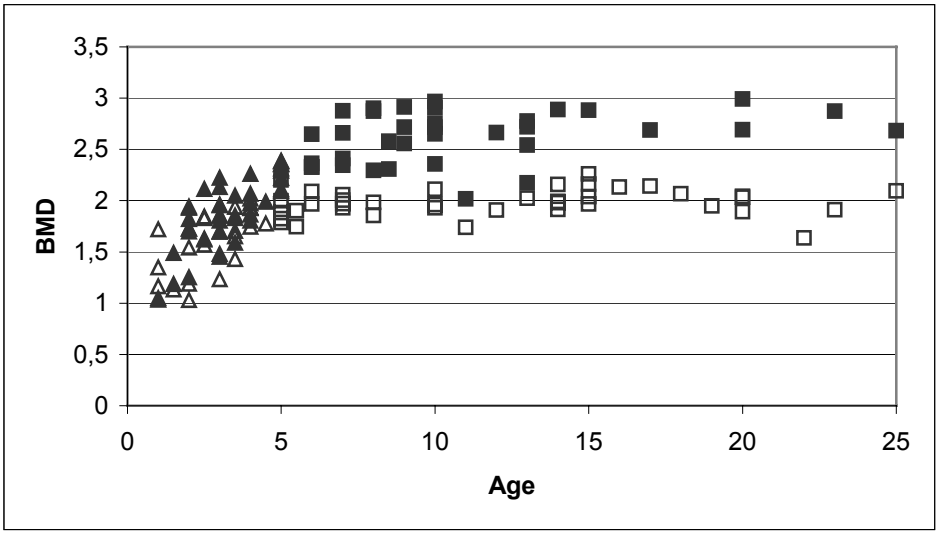

Figure 2.